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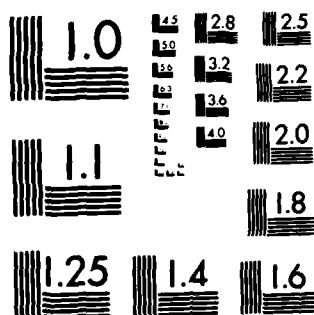
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Annual Report

Daniel J. Stechschulte, M.D.

Herbert B. Lindsley, M.D.

September 1982

(July 1977 - June 1979)

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND  
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD 17-74-C-4136

University of Kansas Medical Center  
College of Health Sciences and Hospital  
Kansas City, KS 66103

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The immunologic response of Sprague-Dawley rats infected with <u>Trypanosoma rhodesiense</u> is manifested by glomerulonephritis with IgM and IgG1 deposition, activation of both the classic and alternate complement proteins and polyclonal B cell activation as detected by antibody production to native DNA. The role of isotype specific immune responses in protective immunity versus immunopathologic tissue injury has not been determined. The nature of the antigens participating in the immune responses has not been clarified but the apparent differences in the evidence and severity of glomerulonephritis in (Cont'd on reverse side)		

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selected inbred strains of rats suggests that specific regulation of the immune response is potentially of value in both immunity and tissue injury. The lack of the IgG antibody response in the sera of inbred strains as detected by unfractionated solubilized T. rhodesiense antigen in ELISA, to differentiate between minimal and moderate glomerulonephritis is puzzling. Possible explanations are that the IgG antibody response has no role in immune complex deposition or that the antigen preparation does not discern subtle changes in the immune response. The apparent relationship between the IgM antibody response and glomerulonephritis in the Buffalo strain is of interest and should lead to a further understanding of immunopathogenic mechanisms in the infection.

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## Summary

The immunologic response of Sprague-Dawley rats infected with Trypanosoma rhodesiense is manifested by glomerulonephritis with IgM and IgG1 deposition, activation of both the classic and alternate complement proteins and polyclonal B cell activation as detected by antibody production to native DNA. The role of isotype specific immune responses in protective immunity versus immunopathologic tissue injury has not been determined. The nature of the antigens participating in the immune responses has not been clarified but the apparent differences in the evidence and severity of glomerulonephritis in selected inbred strains of rats suggests that specific regulation of the immune response is potentially of value in both immunity and tissue injury. The lack of the IgG<sub>a</sub> antibody response in the sera of inbred strains as detected by unfractionated solubilized T. rhodesiense antigen in ELISA, to differentiate between minimal and moderate glomerulonephritis is puzzling. Possible explanations are that the IgG antibody response has no role in immune complex deposition or that the antigen preparation does not discern subtle changes in the immune response. The apparent relationship between the IgM antibody response and glomerulonephritis in the Buffalo strain is of interest and should lead to a further understanding of immunopathogenic mechanisms in the infection.



## FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

Report: July 1, 1977 to December 31, 1979

The work accomplished under this contract can be summarized as follows:

1. The biological importance of the various classes of rat immunoglobulins such as IgG, and its subclasses, IgA, IgM and IgE in the rat are presumably similar to the biologic activities of these immunoglobulin classes in other species. However, the antibody response in the host-defense reaction in the rat infected with trypanosoma rhodesiense has not been characterized. Continuing attempts have been made to identify the various immunoglobulin classes and sub-classes in the rat, and to develop monospecific reagents for each set of molecules. These reagents will permit the assessment of quantitative immunoglobulin levels following Trypanosoma rhodesiense infection and the measurement of the specific antibody response to this organism within each class or sub-class, if the appropriate antigens can be identified. A major problem has existed in separating and identifying the sub-classes of IgG. Therefore in our present work antisera to IgGa may well have some reactivity to IgGb and IgGc but does not react with IgGl which is felt to be a fourth sub-class of the 7-S IgG immunoglobulins in the rat.

Monospecific antisera have been prepared to the IgGl immunoglobulin by absorption with IgG Sepharose. In addition, this molecule has been prepared in pure form so that quantitative radial immunodiffusion studies are feasible. The IgGl response to T. rhodesiense infection in the rat has been quantitated

in relative terms and absolute amounts. The biologic activity of IgG1 is not definitively known. Conflicting reports exist in the literature concerning its ability to fix the complement proteins in the classical pathway (Morse et al. 1968; Jones, 1969). Attempts have been made to utilize monospecific anti-IgG1 and affinity chromatography to selectively isolate a pure preparation of IgG1. To date this has been unsuccessful, possibly due to the fact that the gamma globulin fraction of monospecific antisera have been utilized in the affinity chromatography studies.

Monospecific antisera to IgM and a purified IgM preparation have been prepared permitting us for the first time to obtain quantitative levels of this immunoglobulin during the course of T. rhodesiense infection. This reagent will also be utilized, as described above for IgG1, to extract the IgM immunoglobulins from sera of infected animals and definitely separate and quantitate the 19S from 8S species by combining radial immunodiffusion techniques and sucrose density gradient ultracentrifugation.

As indicated above, the Fc specific antiserum to IgG reacts primarily with IgGa and IgGb but cross reactivity with IgGc is known to exist. It appears that IgGa is the major molecule on a quantitative basis. Additional efforts to separate the IgGa from IgGb and IgGc sub-classes and the generation of monospecific antisera to each sub-class will not be pursued until additional myeloma proteins are obtained from Dr. Bazin. Quantitative

data will be generated recognizing that the results will reflect a mixture of IgGa, IgGb and IgGc.

2. The immune response of rats infected with T. rhodesiense 1886 can be characterized by an increase in the IgG1 and IgM serum immunoglobulin levels associated with hypocomplementemia manifested by activation of both the alternative and classical pathways. In addition these animals develop glomerulonephritis with evidence of immune complex deposition and produce antibody to nucleic acids antigens (Appendix A). The effect of the infection on the immune response in general has been studied and there does not appear to be a marked enhancement of an ongoing antibody response to a selected antigen such as DNP-BGG. This would argue that the hypergammaglobulinemia noted in animals infected with this parasite is in response to parasitic antigens. To date we have been unable to identify these antigens, but the above data directs attention to this abnormality as a fruitful area of investigation.

3. The development of immune complex nephritis in rats infected with T. rhodesiense has been documented (Appendix A). We feel that this observation will provide a useful model for studying specific humoral immune responses and correlating it with the degree of glomerulonephritis and possibly identifying the antigens involved in this manifestation of the infection.

4. It has not been possible to iodinate intact organisms for production of a radiolabeled antigen

capable of reacting with post infection sera. Precipitin lines can be identified as IgGa, IgGb, IgGc, IgGl and IgM. However, radioautography fails to demonstrate any specific radiolabeled antigen binding. In addition to the 25 day post infection sera, 11 day sera have been tested with negative results.

5. Immune response monitored by an enzyme-linked immunosorbent assay (ELISA)

a) Humoral immune response in Trypanosome-infected and immunized rats.

As we were unsuccessful in demonstrating antigen binding activity of sera from infected rats to radioiodinated trypanosomal antigens, we have utilized an alternative assay system with very encouraging results. Use of these assays is growing rapidly, as is apparent from a number of recent reviews (Bidwell et al, 1976; Schuurs and Van Weemen, 1977). With the assistance of Dr. Ken Walls at the Center for Disease Control, Atlanta, Georgia, we have set up an ELISA similar to one already published for trypanosomal antibodies (Voller et al, 1975). The antigen preparation consists of T. rhodesiense 1886 organisms purified by DEAE cellulose chromatography, sonicated and dialyzed. To establish optimal test conditions a serum pool from 25 rats infected with T. rhodesiense 1886 for 18 days was used at the source of primary antibodies. Preliminary experiments established the following working conditions; trypanosomal antigen concentration 5 ug/ml, horseradish peroxidase conjugated to rabbit anti-goat IgG at a final dilution of 1:9600 (obtained from Cappel Laboratories,

Cochranville, Pa) enzyme substrate incubation 60 minutes rather than the 30 minutes recommended by the CDC. Three microtiter plates were tested: Falcon tissue culture plate, Dynatech polyvinyl flexible plate and Dynatech microELISA substrate plate. The last plate showed the greatest discrimination between positive (P) and negative (N) sera over several dilutions and was used for further studies.

In order to demonstrate specificity of binding, plates were sensitized with trypanosomal antigen, BSA or gelatin, all at a concentration of 5 ug/ml. Dilutions of the reference serum pool less than 1/100 final dilution demonstrated non-specific binding of rat IgG to BSA and gelatin. In brief, the ELISA system is performed as follows: trypanosomal antigen is added at a concentration of 5 ug/ml to each well in a microtiter plate followed by incubation at 37° for three hours and 4° overnight. On the day of use, the antigen solutions are removed and the wells filled with rat sera at a final dilution of 1:300 (50ul of a 1:50 dilution, to which is added 250ul of buffer). Voller and associates used a final dilution of 1:600. As second antibodies goat anti-rat IgG (diluted 1:400) were used. Horseradish peroxidase conjugated rabbit anti-goat IgG was at a dilution of 1:9600. With the modifications indicated above, the ELISA system is carried out as previously described (Walls et al, 1977). The contents of the well are read in a Gilford 240 spectrophotometer rapid sampler with a small volume cuvette adapter satisfactory for reading volumes as small as 0.2 ml.

The enzyme immunoassay was utilized to monitor rat antibody responses, extending the preliminary studies outlined above. A crude preparation of soluble, sonicated, dialyzed trypanosomal antigens was used to measure antibody responses of the rat IgM, IgG1 and IgGa classes with respect to (1) time course of infection, (2) inbred strains and (3) immune deposits eluted from kidney tissue. Sera from previously infected outbred rats have been stored at 85°C and will be tested. High and low molecular weigh IgM antibodies will be separated by preparative ultracentrifugation (Stage and Mannik, 1971) for testing in ELISA.

These studies demonstrate the usefulness of the rodent model for studying this infection. It can also be concluded that the complexity of the immune response and the multiple antigens presented during the course of an infection dictate that monospecific reagents for lymphocytes and immunoglobulin molecules be available for measuring the response to purified T. rhodesiense antigens.

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